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# RECOGNITION OF PATHOGENIC MICROBES IN THE EXTERNAL MEDIUM WITH CONSIDERATION OF THE VARIABILITY OF THEIR PROPERTIES

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The recognition of changed microbes and the establishment of their role in the etiology and epidemiology of infectious diseases is a recurrent task of microbiologists.

The comparative study of the antigenic structure of typical and atypical microbes which are being isolated both under natural conditions, and also experimentally can serve as a basis for the establishment of the nature of the so-called atypical microbes and their bond with the typical representatives of the given species.

The material being presented sheds light on the question of the possibility of the use of data concerning variability for the indication of pathogenic microbes in the external medium.

In the small village of N. there had annually taken place isolated cases of typhoid fever. In May 1954 in this small village a school boy became sick with typhoid fever. In searching for the possible source of the infection, attention was directed toward the water in the school tank. As a result of the analysis of the water conducted according to the Fikher method, a culture was isolated which did not cleave lactose on a Russel medium and fermented glucose with the formation of acid without gas.

The culture consisted of gram negative bacilli which did not agglutinate specific antityphoid serum, but which gave a clear-cut agglutination on a slide with specific serum for the B-20 strain obtained in the Gor'kiy Institute of Vaccines and Serums via the cultivation of the typhoid fever bacillus in water(Blokhina).

Strain B-20 consists of gram negative mobile bacilli, cleaving among the carbohydrates of the short variegated series only glucose (with the formation of acid). The culture does not agglutinate typhoid fever serums.

In the presence of passages in serum bouillon the reversion of this strain to the typical typhoid fever bacillus was noted.

The culture which was isolated from the water of the school tank by the local sanitary-bacteriological laboratory and which agglutinated the B-20 serum, was sent to the Gor'kiy Institute of Vaccines and Serums for identification.

In the institute, with the object of determining the homogeneity of the population and of the cultural properties, the strain was seeded in a dish with Endo medium and bactoagar Zh [J, G]. After a day of raising at 37° on Endo medium and after 2 days on bacteagar Zh there appeared a growth of homogeneous tender colonies with a rosy tinge. Five colonies each were taken from both media on an agar slant. In a check it turned out that they all consisted of gram negative immobile bacilli which in the short variegated series fermented glucose, mannitol, and sucrose with the formation of acid without gas. The cultures did not form indole and hydrogen sulfide and did not cause the lysis of typhoid fever phage. The agglutination reaction with specific and polyvalent antityphoid fever serum was negative in all cultures. At the same time cultures from all 10 colonies

agglutinated up to 1/4 titer of serum for strain B-80. The cultures did not agglutinate antidysentery sera.

Passages for the course of a month on diverse nutrient media (meat-peptone agar, serum bouillon, bile bouillon) did not lead to a change in the properties of the cultures. With the object of obtaining a reversion to the typical typhoid fever bacillus, we administered 500 million of these microbes intraperitoneally to mice. At 3 days after the infection the mouse was killed and from its organs -- the liver, spleen, inguinal lymphatic nodes, and blood from the heart -- a seeding in a dish with meat-peptone agar was made. The seedings of the liver, spleen, and blood remained sterile. From the inguinal lymphatic nodes, along with the unchanged cleaved glucose and mannitol with the formation of acid and formed hydrogen sulfide. The culture was agglutinated up to titer by antityphoid fever serum, and contained Vi-antigens. The experiments with the infection of mice with the strain isolated from the water were repeated. In the repeated experiments we also obtained reversion of the strain being studied to the typical typhoid fever bacillus. As a rule typical typhoid fever microbes were isolated from the organs in those cases in which the mice perished, seedings from the organs of mice which survived infection proved to be either sterile, or the unchanged starting culture was isolated from them.

The virulence and immunogenicity of the starting culture isolated from water and designated No 1 was studied on mice. The strain proved to be avirulent -- the culture did not kill mice in a dose of up to 5 billion microbial bodies -- and was nonimmunogenic; immunization with it provided only 14% survivability of the mice. At the same time the DoI of the strain which had reverted was equal to 250 million

microbial bodies. Via the intravenous immunization of a rabbit to strain No 1 isolated from water, a serum with a titer of 1:1,600 was prepared. The strain which had reverted agglutinated this serum up to 1/8 titer.

## CONCLUSIONS

1. A nontypical culture was isolated from the water of a tank in a school where there had taken place a case of disease with typhoid fever which culture later on turned into the typical typhoid fever bacillus.

2. The recognition of this atypical culture proved to be possible only thanks to the application of a specially obtained serum acting against the variant of the typhoid fever bacillus.